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A QUANTITATIVE ANALYSIS OF THE ANNUAL TESTICULAR CYCLE OF THE BRITTLE-STAR *AMPHIPHOLIS KOCHII* BY MEANS OF AUTORADIOGRAPHIC INVESTIGATION

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Abstract

This paper concerns the annual testicular cycle of the brittle-star *Amphipholis kochii* (Echinodermata: Ophiuroidea). An autoradiographic study was applied for analysis of the seasonal changes in the number of spermatogenic cells, the duration of spermatogenesis and the spermatocyte production rate. The annual testicular cycle can be divided into five phases; spermatogenesis occurs during phases 2 to 4 (November to June). Spermatogenesis during phases 3 and 4 (March to June) was sensitive to the sea water temperature as an external environment, while that during phase 2 (November to February) was insensitive to it. The water temperature directly controls the speed of the later phase of spermatogenesis (from spermatocyte to spermatozoon), but it is not able to control the spermatocyte production rate. It is therefore suggested that the water temperature plays an important role in spermatogenesis during phases 3 and 4 by controlling the speed of the later phase of spermatogenesis, and that other environmental factor(s) controlling the spermatocyte production rate are likely to be important to spermatogenesis during phase 2.

Introduction

Although many detailed studies have been made on the reproductive cycle of echinoderms, especially on that of the echinoids, the strict relationship between the reproductive cycle and environmental changes is still unclear. Based upon a superficial correlation between them, several authors have suggested that the reproductive periodicities might be controlled by environmental factors such as water temperature (Boolootian, 1966; Pearse and Phillips, 1968; Chatlynne, 1969; Pearse, 1969a, b, 1970; Lessios, 1981), salinity (Lessios, 1981), lunar periodicity (Pearse, 1975), tidal change (Pearse, 1972), food supply (Fuji, 1960; Pearse and Giese, 1966; Pearse, 1969a, b; Gonor, 1973), and photoperiod (Holland, 1967; Pearse, 1969a). To date, however, experimental studies to clarify the relationship between the reproductive cycle and these environmental factors have been confined to only a few investigations (Cochran and Engelmann, 1975; Pearse, 1981; Pearse and Eernisse, 1982).

Concerning the reproductive cycle of ophiuroids, several examinations have been published until now, but the effect of the external factors on gametogenesis has never been experimentally confirmed (Patent, 1969; Fenaux, 1970, 1972; Lönning, 1976; Tyler, 1977; Hendler, 1979; Tyler and Gage, 1980, 1982; Gage and Tyler, 1982). In our previous paper, we described the gross features of the annual reproductive cycle of the brittle-star *Amphipholis kochii* (Iwata and Yamashita, 1982), but detailed examinations on the reproductive cycle and the environmental factors still remain. Therefore, a quantitative analysis of the reproductive cycle and an

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experiment to clarify the relationship between the reproductive cycle and the sea water temperature as an environmental factor are carried out in this study.

Materials and Methods

The brittle-stars, *Amphipholis kochii*, used in this study were collected between the tidemarks at Abuta on the Pacific coast of south-western Hokkaido, Japan, at monthly intervals from June 1979 through August 1980. Several animals from each collection were fixed in Bouin's solution for ordinary histological preparations as soon as they had been transported to the laboratory, while the remainder were maintained until use.

Calculation for the number of cells in testis

The number of cells in the testis was calculated by the following procedure, based on the method of Holland *et al.* (1975):

1) Assuming that the testis is a solid cylinder with a constant diameter, the testicular volume was calculated according to its length and diameter.

2) Under the light microscope, the histological cross section of a given testis was divided into four regions, each of which is occupied mainly by spermatogonia, spermatocytes, spermatozoa together with spermatids, and others, respectively. The boundary of each region was clearly observable by histological examination (Figs. 1C, D). In order to assess the percentage of each cell population in a cross section, the histological cross section of the testis was projected onto an aluminum foil by means of *camera lucida*. The aluminum foil was cut along the boundary of each cell region and weighed by a chemical balance. The weight of each aluminum foil was divided by the total weight.

3) To estimate the volume of one spermatogonium, spermatocyte or spermatozoon (or spermatid), an arbitrary number of cells in each cell population was projected onto an aluminum foil at a magnification of 920×. The projected foil was then weighed, and its weight was divided by the weight of the standard aluminum foil of 920 mm × 920 mm for conversion of weight to area. The resulting area was divided by the number of the cells projected to yield the area occupied by one cell. From this area, the volume of one cell was calculated on the assumption that the cell was adequately represented as a sphere. The average volume of each cell type after several measurements was as follows:

$$\text{Spermatogonium: } 1.0 \times 10^{-6} \text{ mm}^3$$

$$\text{Spermatocyte: } 3.6 \times 10^{-7} \text{ mm}^3$$

$$\text{Spermatozoon (Spermatid): } 2.4 \times 10^{-8} \text{ mm}^3$$

4) In order to calculate the cell number of each cell population from a given testis, the testicular volume was multiplied by the percentage of each cell population and then divided by the volume of the one cell. Three arbitrary testes were measured for an individual, and the average number of cells was used for the data.

To assess the number of the amoeboid cells (the somatic cells in the testis), the foregoing method could not be employed because the locality of these cells in the testis was not cylindrical but very irregular (Fig. 1E). Thus, the area of the amoeboid cell clusters in a cross section was used for estimating the number of the amoeboid

cells. It was accomplished as follows: The outline of the clusters of the amoeboid cells in a cross section was projected onto an aluminum foil at a magnification of 194×, and the weight of the foil obtained was divided by the weight of the foil of 194 mm square. The average area after three measurements was used for the data.

Experiment on temperature influence

To examine the influence of sea water temperature on spermatogenesis, some brittle-stars collected in February, April and May were separated into two groups, each containing about five animals; one group, as a control, was kept in the aquarium at the same temperature as that of the sea water at the collection site, while the other group was kept at a higher or lower temperature than that. In this case, the day length at the collection site was roughly maintained in experiment. Eighteen days later, the animals were fixed, and the number of the spermatogenic cells were calculated by the aforementioned procedure.

Autoradiography

The brittle-stars collected during the season from November through June were injected intracoelomically with 5 μCi of ^{3}H-thymidine (specific activity, 42 Ci/m mol: New England Nuclear, Boston). The ^{3}H-thymidine was diluted for use with one part of sea water. The injected animals were kept in the aquaria at various water temperatures including the same temperature at which the samples were collected. At intervals after the injection of 1 h, 1 day, and days 3, 5, 7, 10, 14, 18, 22, 26 and 30, several animals were fixed in Bouin's solution, embedded in paraffin (Tissue Prep; Fischer Scientific Co., New Jersey), and sectioned in pieces 2–3 μm thick. After being deparaffinized, the slides were extracted with cold 2% perchloric acid and dipped in half-diluted NR-M2 nuclear emulsion (Konishiroku Photo Ind., Tokyo) at 45°C; they were then exposed for 10 days at 4°C. The slides were stained with Delafield's hematoxylin and eosin after the photographical development following the application of emulsion.

Electron microscopy

The testes were prefixed with 5% glutaraldehyde in 75% sea water, washed in 150% sea water, and post-fixed with 1% OsO_4 in 75% sea water. The fixed testes were dehydrated with acetone and embedded in Epon 812. Ultrathin sections were cut with glass knives on a Porter-Blum MT-1 ultramicrotome, stained with uranyl acetate and lead citrate, and observed in a JEOL 100S electron microscope.

Results

Histology of the testis

The testes in the disk are little sacs attached to the coelomic wall of the bursae, arranged in a row (Figs. 1A, B). In the testis, zonation of spermatogonia, spermatocytes and spermatozoa together with spermatids can be recognized (Figs. 1C, D). Mature spermatozoa gather to form large clusters in the central portion of the testis (Fig. 1C). The somatic cells in the testis known as amoeboid cells (Iwata and Yamashita, 1982) are usually found during the formation assembly in the sperm mass (Fig. 1E).

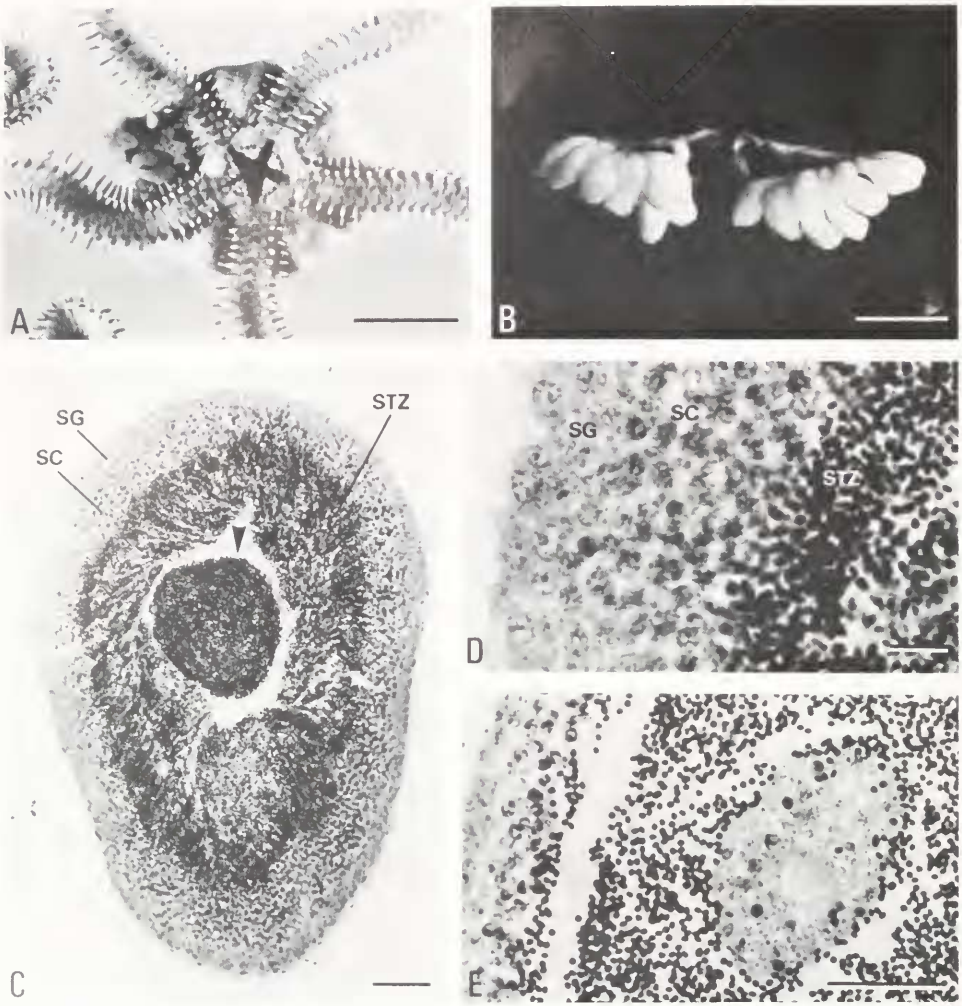

FIGURE 1. A: Photograph of *Amphipholis kochii* from the ventral view, partly dissected to show the attachment and arrangement of the testes. Scale = 5 mm. B: Photograph of the testes isolated from one inter-radial part. Scale = 1 mm. C: Histological cross section of the testis, showing a zonation of spermatogonia (SG), spermatocytes (SC) and spermatozoa together with spermatids (STZ). Scale = 100 μm. D: High magnification of the testis in Figure 1C. From the testicular wall to the inside, spermatogonia (SG), spermatocytes (SC) and spermatozoa together with spermatids (STZ) form zones. Scale = 50 μm. E: The amoeboid cell cluster in the sperm mass. Scale = 50 μm.

Annual cycle of spermatogenesis

Figure 2 gives monthly fluctuations in the number of the spermatogenic cells, the area of the amoeboid cell clusters and the sea water temperature at the collection site. As a whole, the following five phases are distinguishable in the annual testicular cycle.

Phase 1: August to October. Spermatogenesis is arrested, and the spermatocytes are absent. A small number of the relict spermatozoa are ingested by the amoeboid cells forming large clusters.

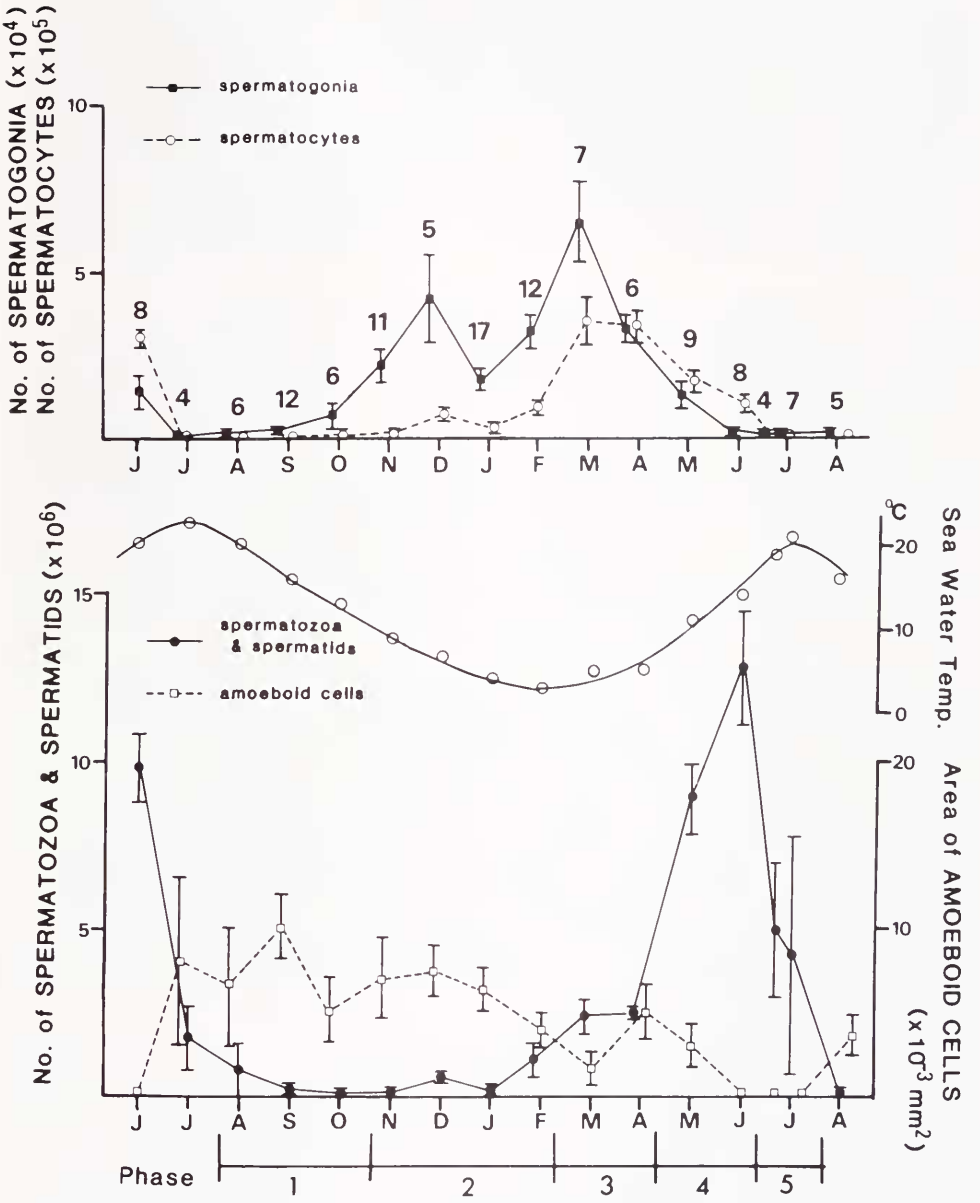

FIGURE 2. Monthly variations in the number of the spermatogenic cells and in the area of the amoeboid cell clusters in *Amphipholis kochii*. Changes of the sea water temperature at the collection site are also indicated. The numbers indicated over the upper graph show the number of the animals used each month. Average ± standard error.

Phase 2: November to February. Spermatogenesis recommences, but the number of the spermatozoa is still very small. The clusters of the amoeboid cells are still large.

Phase 3: March to April. The spermatocytes increase abruptly in number, and the number of the spermatozoa and spermatids becomes larger than in phase 2, but

TABLE I

Number of the spermatocytes and the spermatozoa with spermatids in Amphipholis kochii

	Sea water temperature (°C)	No. of spermatocytes ($\times 10^4$)	No. of spermatozoa & spermatids ($\times 10^5$)
February	3 (control)	7.3 ± 2.0	10.1 ± 4.6
	13	4.7 ± 1.0	10.8 ± 3.1
April	5 (control)	33.6 ± 5.2	25.3 ± 1.4
	9	29.1 ± 6.7	62.6 ± 14.9
May	11 (control)	9.7 ± 3.5	93.5 ± 12.1
	4	41.4 ± 14.5	70.0 ± 31.5

The animals were kept at a higher or lower temperature than that at the collection site in February, April and May.

Average ± standard error.

much smaller than in phase 4. During this period, the sea water temperature at the collection site is still as low as during phase 2.

Phase 4: May to June. The sea water temperature rises rapidly. The number of the spermatozoa and spermatids increases abruptly, reaching maximum in June.

Phase 5: July. A sudden decrease in the number of the spermatozoa and spermatids occurs as a result of spawning (Iwata and Yamashita, 1982).

Influence of sea water temperature on spermatogenesis

Table I shows the result obtained from the experiment to examine the influence of sea water temperature on spermatogenesis. In February (phase 2), there was no significant difference in the number of the spermatogenic cells between the control and experimental groups. In April (phase 3) and May (phase 4), however, an apparent difference between the two groups was detected. In April, the number of the spermatozoa and spermatids in the animals which had been kept at higher water temperature was significantly larger than that in the control ($P < 0.05$, t-test). In May, the number of the spermatozoa and spermatids at the lower temperature was smaller than that in the control. It was also notable that in May the number of the spermatocytes at lower temperature was much larger than in the control ($P < 0.1$).

These results indicate that the high water temperature accelerates the accumulation of spermatozoa and that the low water temperature inhibits it in phases 3 and 4. In phase 2, however, the water temperature has no influence on the accumulation of spermatozoa.

Duration of spermatogenesis at various temperatures

Figure 3 shows autoradiograms through the testis of the brittle-star *Amphipholis kochii* after the injection of ^{3}H-thymidine. Even 1 h after the injection, the spermatogonia and spermatocytes were heavily labeled, but the spermatids and spermatozoa were not labeled at all (Fig. 3A). Several days after the injection, the spermatids or the spermatozoa became labeled (Figs. 3B, C). This feature shows the occurrence of a possible transformation of the labeled spermatogonia or spermatocytes to the spermatids or spermatozoa, *in vivo*. Finally, the labeled spermatozoa were found in the cytoplasm of the amoeboid cells (Fig. 3D). Under the electron microscope, we could easily detect these spermatozoa ingested by the amoeboid cells after a phagocytic action (Fig. 4). Based upon these results, the duration necessary

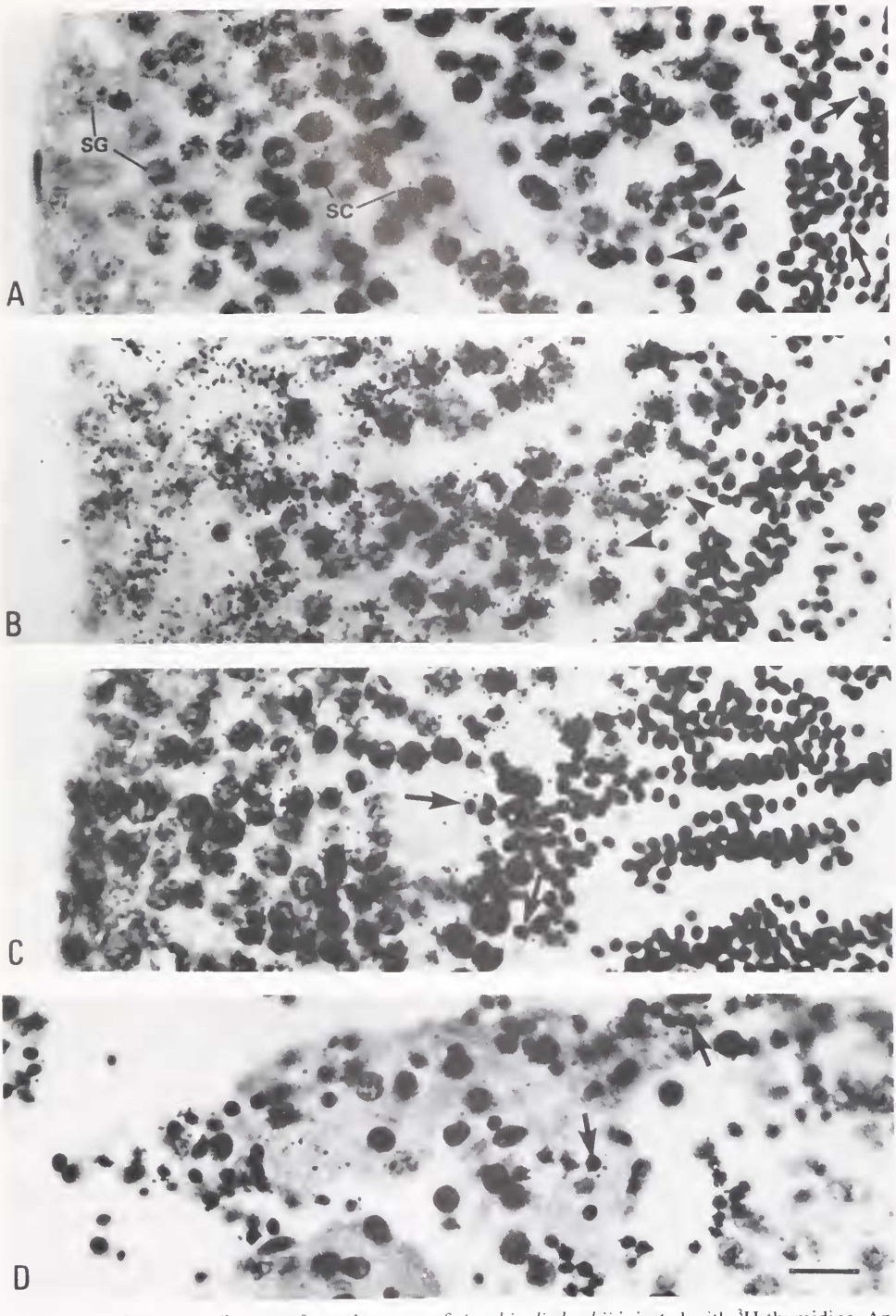

FIGURE 3. Autoradiograms from the testes of *Amphipolis kochii* injected with ^{3}H-thymidine. Animals were collected at various seasons and reared under experimental conditions. Figures 3A–C, testes in March reared at 5°C; Figure 3D, testis in November reared at 16°C. Scale = 10 μm. A: 1 hour after injection. The silver grains are heavily developed over the spermatogonia (SG) and spermatocytes (SC), but none over the spermatids (arrowheads) and spermatozoa (arrows). B: 14 days after injection. Grains are found in the spermatids (arrowheads), but not in the spermatozoa. C: 18 days after injection. The spermatozoa (arrows) have been labeled. D: 10 days after injection. The labeled spermatozoa (arrows) have been ingested in the cytoplasm of the amoeboid cells forming a cluster.

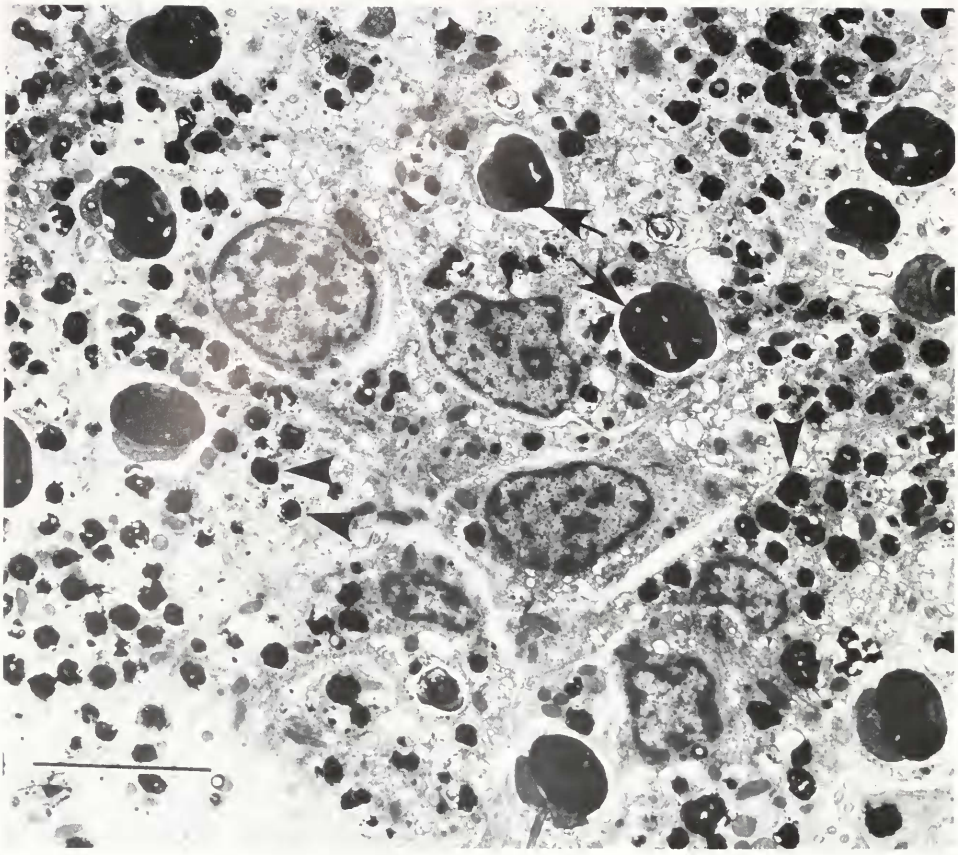

FIGURE 4. Electron micrograph of the amoeboid cells of *Amphipholis kochii* forming a cluster in the sperm mass. Note the mature spermatozoa (arrows) ingested by the amoeboid cells by a phagocytic activity. In the cytoplasm of the amoeboid cells many residual bodies which are probably derived from degenerate spermatozoa are also seen (arrowheads). Scale = 5 μm.

for the transformation from the spermatocyte to the spermatid or to the spermatozoon was determined by detecting the first appearance of the labeled spermatid or spermatozoon in the autoradiograms.

Table II gives the duration of spermatogenesis at various water temperature as well as at the same temperature as that at the collection site. The duration of spermatogenesis faithfully correlated to the water temperature at which the animals had been kept. The same experimental temperature for cultivation yielded the same duration of spermatogenesis irrespective of whether the animals had been collected at different months. In order to demonstrate the relationship between the duration of spermatogenesis and the water temperature more closely, the durations obtained in each month in Table II were plotted against the water temperature (Fig. 5). As the water temperature became higher, the duration of spermatogenesis became shorter, and *vice versa*. A close relationship between the water temperature and the duration of spermatogenesis was more apparent in the early stage of spermatogenesis, from spermatocyte to spermatid, than in the later stage, from spermatid to spermatozoon or spermiogenesis.

TABLE II

Duration of spermatogenesis in Amphipholis kochii *at various water temperatures*

	Nov		Dec	Jan	Feb		Mar		Apr		May		Jun	
Sea water temperature (°C) (collection site)	9		7	4	3		5		5		11		14	19
Sea water temperature (°C) (experiment)	9	16	7	4	3	13	5	18	5	9	11	4	14	19
Duration of spermatogenesis (days)														
spermatocyte—spermatid	7	5	10	18	22	5	14	3	14	7	7	22	5	3
spermatocyte—spermatozoa	14	7	14	22	30	10	18	5	18	14	10	30	7	5
spermatid—spermatozoa	7	2	4	4	8	5	4	2	4	7	3	8	2	2

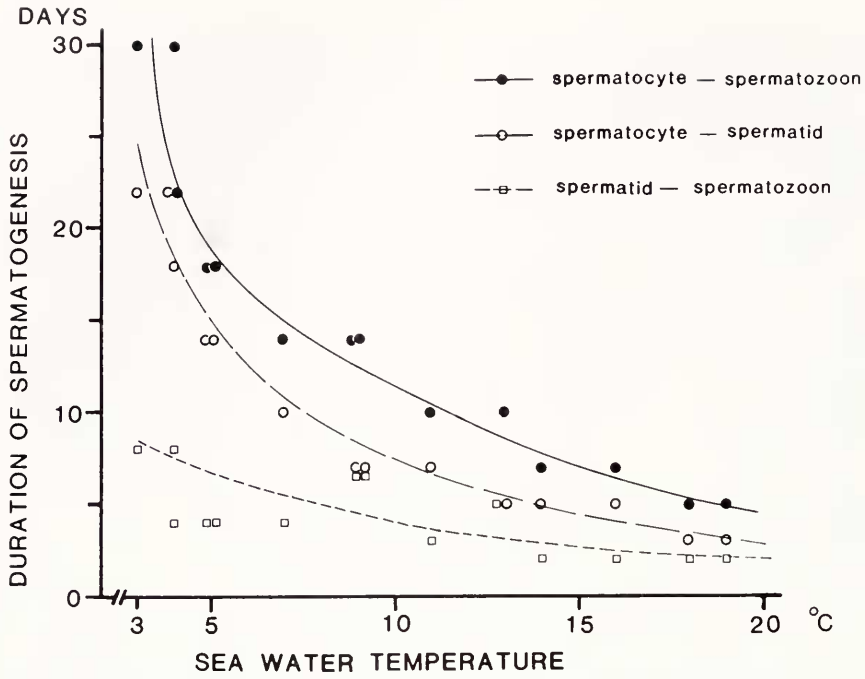

FIGURE 5. Correlation between the duration of spermatogenesis of *Amphipholis kochii* and the sea water temperature. The animals were reared at various experimental temperatures and the duration of spermatogenesis in each experiment was then measured.

Seasonal changes in the spermatocyte production rate (SPR)

To estimate the SPR per day throughout each month, the number of the spermatocytes (Fig. 2) was divided by the duration of the spermatocytic stage (the duration from spermatocyte to spermatid, Table II). Table III shows the seasonal changes in the SPR. It was apparent that the seasonal changes in the SPR had two distinct periods, one from November to February (phase 2) and the other from March to June (phases 3 and 4). The SPR in phases 3 and 4 was about six times higher than that in phase 2. The SPR was high in phase 3 though the sea water temperature was noticeably low at this season (Fig. 2). In phase 2, however, the SPR

TABLE III

The spermatocyte production rate in Amphipholis kochii *during the season from November to June*

	Phase 2				Phase 3		Phase 4	
	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
No. of spermatocytes ($\times 10^4$) (N)	1.4	7.2	3.3	9.2	34.6	33.6	17.3	9.7
Duration of spermatocytic stage (days) (D)	7	10	18	22	14	14	7	5
N/D	0.2	0.7	0.2	0.4	2.5	2.4	2.5	1.9
Spermatocyte production rate (per day)	1.0	3.5	1.0	2.0	12.5	12.0	12.5	9.5

The rate in each month is represented as a ratio to the rate obtained in November.

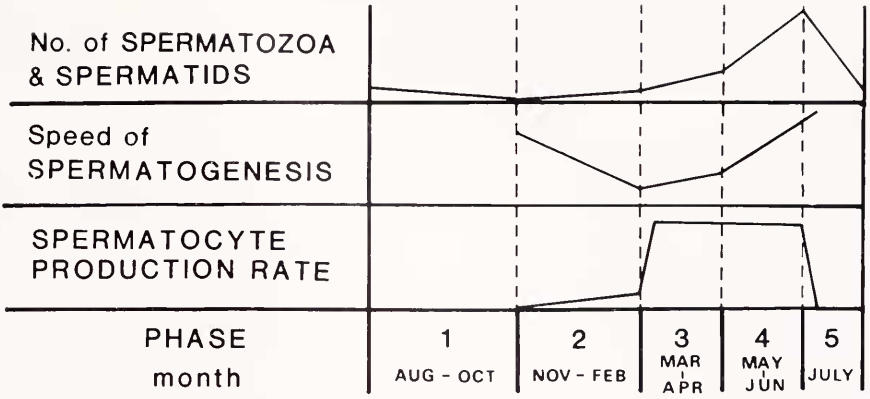

FIGURE 6. Seasonal tendencies in the number of the spermatozoa and spermatids, the speed of spermatogenesis and the spermatocyte production rate, summarized from Figure 3 and Tables II and III.

was very low even at almost the same or higher water temperature than that in phase 3 (Fig. 2). These results seemed to indicate clearly that the water temperature was not able to control the SPR.

DISCUSSION

The present study demonstrates a close correlation between the duration of spermatogenesis of the brittle-star *Amphipholis kochii* and the water temperature as an external environment, both under normal and experimental conditions. Seasonal changes in the annual testicular cycle and the factors controlling spermatogenesis are summarized in Figure 6. Based upon Figure 6, we suggest the following control mechanisms for spermatogenesis during phases 2 to 4. Phase 2: The sluggish accumulation of the spermatozoa in this phase is probably due to the low spermatocyte production rate. In this phase, the water temperature influences neither the accumulation of the spermatozoa nor the spermatocyte production rate (Tables I and III). The water temperature should not therefore contribute to the control of spermatogenesis during this phase. Phase 3: Owing to the high spermatocyte production rate, the accumulation of the spermatozoa becomes active, but it is slower than that in the next phase. This situation is presumably due to the slow speed of spermatogenesis resulting from the low sea water temperature at this season (Figs. 2, 5). This explanation is consistent with the experimental results that the number of the spermatozoa grew larger when the animals were kept during phase 3 at a higher water temperature and that it decreased during phase 4 when the animals were kept at a lower temperature (Table I). The increase of the spermatocytes in the animals kept at a lower temperature in phase 4 (Table I) is therefore probably due to the slow speed of spermatogenesis caused by the low water temperature. Phase 4: The spermatocyte production rate is high and the speed of spermatogenesis is fast owing to the high sea water temperature, resulting in a rapid accumulation of the spermatozoa.

With respect to the water temperature as an external factor on the control of the annual reproductive cycle, the testicular cycle of *Amphipholis kochii* can be divided into two distinct periods: the temperature-insensitive period during the season from November to February (phase 2) and the temperature-sensitive period from March to June (phases 3 and 4). The water temperature plays an important role as a key factor on spermatogenesis during phases 3 and 4, while other envi-

ronmental factors such as photoperiod or food supply are likely to be key factor(s) on spermatogenesis during phase 2. Further studies to examine the effect of day length or food supply on spermatogenesis, especially during phase 2, seem to be necessary.

Concerning the seasonal changes in the speed of spermatogenesis, Holland and Giese (1965) carried out an autoradiographic study on the testis of the echinoid *Strongylocentrotus purpuratus,* and obtained the result that the duration of spermatogenesis remained constant throughout the annual cycle. An explanation for Holland and Giese's result, in contrast with that of this study, might be that they measured the duration of spermatogenesis at relatively constant water temperatures throughout the annual cycle.

The possible participation of the somatic cells in the control of the spermatozoa production rate cannot be ignored. By means of an autoradiographic investigation, Holland and Giese (1965) revealed the ingestion of newly-formed spermatozoa by the somatic cells in the testis of the echinoid. The present study also demonstrates that the amoeboid cells (the somatic cells) in the testis of *Amphipholis kochii* are able to ingest the newly-formed spermatozoa as well as the relict ones by phagocytic action (Fig. 4). Judging from the presence of large clusters of amoeboid cells during phase 2, we might be allowed to assume that the phagocytic activity of the amoeboid cells upon the newly-formed spermatozoa contributes partly to a slow spermatozoa accumulation in phase 2.

The duration of spermatogenesis has been measured in various animal phyla, and it is certain that marine invertebrates have a relatively high speed of spermatogenesis in comparison with mammals and insects (see Table 9 in Roosen-Runge, 1977). The present measurement also reveals that the spermatogenesis of the brittle-star is of a relatively short duration. This short duration may affect the mode of spermatogenesis. For example, the spermatogenesis of the present species is very peculiar in that the acrosome formation is initiated in the spermatogonium (Yamashita and Iwata, 1983). This precocious production of the acrosome might be related to the short duration of spermatogenesis insufficient for the production of acrosomal material during spermiogenesis. The similar phenomenon has also been reported in the mussel, in which the acrosome formation is initiated in the spermatogonium (Longo and Dornfeld, 1967) and the duration of spermatogenesis is relatively short (Kelley *et al.,* 1982). On the other hand, it is known that the sea urchin has a short period of spermatogenesis (Holland and Giese, 1965) but that the acrosome formation is initiated in the spermatid (Longo and Anderson, 1969). This contrasting situation between the sea urchin, brittle-star, and mussel may be explained by the fact that the acrosome of the sea urchin is small when compared with that of the brittle-star and mussel (Dan, 1967; Yamashita and Iwata, 1983). Although the spermatogenesis of the sea urchin is of a short duration, it may be sufficient for the production of such a small acrosome during the spermatid stage.

It is very interesting to note that the duration of spermiogenesis appears to be independent of temperature (Fig 5). In this respect, Egami and Hyodo-Taguchi (1967) reported that the duration of spermiogenesis in the fish *Oryzias latipes* at 25°C and 15°C showed no significant difference. These observations allow us to suggest that spermiogenesis mainly depends on internal factors.

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LITERATURE CITED

BOOLOOTIAN, R. A. 1966. Reproductive physiology. Pp. 561–613 in *Physiology of Echinodermata.* Interscientific Publ., New York.

CHATLYNNE, L. G. 1969. A histochemical study of oogenesis in the sea urchin, *Strongylocentrotus purpuratus. Biol. Bull.* **136:** 137–184.

COCHRAN, R. C., AND F. ENGELMANN. 1975. Environmental regulation of the annual reproductive season of *Strongylocentrotus purpuratus* (Stimpson). *Biol. Bull.* **148:** 393–401.

DAN, J. C. 1967. Acrosome reaction and lysins. Pp. 237–293 in *Fertilization.* Vol. 1. C. B. Metz and A. Monroy, eds. Academic Press, New York.

EGAMI, N., AND Y. HYODO-TAGUCHI. 1967. An autoradiographic examination of rate of spermatogenesis at different temperatures in the fish, *Oryzias latipes. Exp. Cell Res.* **47:** 665–667.

FENAUX, L. 1970. Maturation of the gonads and seasonal cycle of the planktonic larvae of the ophiuroid *Amphiura chiajei* Forbes. *Biol. Bull.* **138:** 262–271.

FENAUX, L. 1972. Seasonal variations in the gonads of *Ophioderma longicauda* (Retzius), Ophiuroidea. *Int. Revue Gesamten. Hydrobiol.* **57:** 257–262. [In French with English summary]

FUJI, A. 1960. Studies on the biology of the sea urchin. III. Reproductive cycle of two sea urchins, *S. nudus* and *S. intermedius,* in southern Hokkaido. *Bull. Fac. Fish. Hokkaido Univ.* **11:** 49–57.

GAGE, J. D., AND P. A. TYLER. 1982. Growth and reproduction of the deep-sea brittle star *Ophiomuseum lymani* Wyville Thomson. *Oceanol. Acta* **5:** 73–83.

GONOR, J. J. 1973. Reproductive cycle in Oregon populations of the echinoid, *Strongylocentrotus purpuratus* (Stimpson). I. Annual gonadal growth and ovarian gametogenetic cycles. *J. Exp. Mar. Biol. Ecol.* **12:** 45–64.

HENDLER, G. 1979. Reproductive periodicity of ophiuroids (Echinodermata: Ophiuroidea) on the Atlantic and Pacific coasts of Panama. Pp. 145–156 in *Reproductive Ecology of Marine Invertebrates.* S. E. Stancyk, ed. University of South Carolina Press, Columbia, South Carolina.

HOLLAND, N. D. 1967. Gametogenesis during the annual reproductive cycle in a cidaroid sea urchin (*Stylocidaris affinis*). *Biol. Bull.* **133:** 578–590.

HOLLAND, N. D., AND A. C. GIESE. 1965. An autoradiographic investigation of the gonads of the purple sea urchin (*Strongylocentrotus purpuratus*). *Biol. Bull.* **128:** 241–258.

HOLLAND, N. D., J. C. GRIMMER, AND H. KUBOTA. 1975. Gonadal development during the annual reproductive cycle of *Comanthus japonica* (Echinodermata: Crinoidea). *Biol. Bull.* **148:** 219–242.

IWATA, F., AND M. YAMASHITA. 1982. Annual reproductive cycle of the brittle-star *Amphipholis kochii* (Echinodermata: Ophiuroidea), with special reference to the growth pattern of oocytes. *Publ. Seto Mar. Biol. Lab.* **27:** 143–153.

KELLEY, R. N., M. J. ASHWOOD-SMITH, AND D. V. ELLIS. 1982. Duration and timing of spermatogenesis in a stock of the mussel *Mytilus californianus. J. Mar. Biol. Ass. U. K.* **62:** 509–519.

LESSIOS, H. A. 1981. Reproductive periodicity of the echinoids *Diadema* and *Echinometra* on the two coasts of Panama. *J. Exp. Mar. Biol. Ecol.* **50:** 47–61.

LONGO, F. J., AND E. ANDERSON. 1969. Sperm differentiation in the sea urchins *Arbacia punctulata* and *Strongylocentrotus purpuratus. J. Ultrastruct. Res.* **27:** 486–509.

LONGO, F. J., AND E. J. DORNFELD. 1967. The fine structure of spermatid differentiation in the mussel, *Mytilus edulis. J. Ultrastruct. Res.* **20:** 462–480.

LÖNNING, S. 1976. Reproductive cycle and ultrastructure of yolk development in some echinoderms from the Bergen area, western Norway. *Sarsia* **62:** 47–72.

PATENT, D. H. 1969. The reproductive cycle of *Gorgonocephalus caryi* (Echinodermata: Ophiuroidea). *Biol. Bull.* **136:** 241–252.

PEARSE, J. S. 1969a. Reproductive periodicities of Indo-Pacific invertebrates in the Gulf of Suez. I. The echinoids *Prinocidaris baculosa* (Lamarck) and *Lovenia elongata* (Gray). *Bull. Mar. Sci.* **19:** 323–350.

PEARSE, J. S. 1969b. Reproductive periodicities of Indo-Pacific invertebrates in the Gulf of Suez. II. The echinoid *Echinometra mathaei* (De Blainville). *Bull. Mar. Sci.* **19:** 580–613.

PEARSE, J. S. 1970. Reproductive periodicities of Indo-Pacific invertebrates in the Gulf of Suez. III. The echinoid *Diadema setosum* (Leske). *Bull. Mar. Sci.* **20:** 697–720.

PEARSE, J. S. 1972. A monthly reproductive rhythm in the diadematid sea urchin *Centrostephanus coronatus* Verrill. *J. Exp. Mar. Biol. Ecol.* **8:** 167–186.

PEARSE, J. S. 1975. Lunar reproductive rhythms in sea urchins: A review. *J. Interdiscipl. Cycle Res.* **6:** 47–52.

PEARSE, J. S. 1981. Synchronization of gametogenesis in the sea urchins *Strongylocentrotus purpuratus* and *S. franciscanus*. Pp. 53–68 in *Advances in Invertebrate Reproduction*. W. H. Clark, Jr. and T. S. Adams, eds. Elsevier North Holland Inc., New York.

PEARSE, J. S., AND D. J. EERNISSE. 1982. Photoperiodic regulation of gametogenesis and gonadal growth in the sea star *Pisaster ochraceus*. *Mar. Biol.* **67:** 121–125.

PEARSE, J. S., AND A. C. GIESE. 1966. Food, reproduction and organic constitution of the common antarctic echinoid *Sterechinus neumayeri* (Meissner). *Biol. Bull.* **130:** 387–401.

PEARSE, J. S., AND B. F. PHILLIPS. 1968. Continuous reproduction in the Indo-Pacific sea urchin *Echinometra mathaei* at Rottnest Island, western Australia. *Aust. J. Mar. Freshwater Res.* **19:** 161–172.

ROOSEN-RUNGE, E. C. 1977. Spermatogonia and kinetics of spermatogenesis. Pp. 133–144 in *The Process of Spermatogenesis in Animals*. Cambridge Univ. Press, London.

TYLER, P. A. 1977. Seasonal variation and ecology of gametogenesis in the genus *Ophiura* (Ophiuroidea: Echinodermata) from the Bristol Channel. *J. Exp. Mar. Biol. Ecol.* **30:** 185–197.

TYLER, P. A., AND J. D. GAGE. 1980. Reproduction and growth of the deep-sea brittle star *Ophiura ljungmani* (Cyman). *Oceanol. Acta* **3:** 177–185.

TYLER, P. A., AND J. D. GAGE. 1982. The reproductive biology of *Ophiacantha bidentata* (Echinodermata: Ophiuroidea) from the Rockall Trough. *J. Mar. Biol. Ass. U. K.* **62:** 45–55.

YAMASHITA, M., AND F. IWATA. 1983. Ultrastructural observations on the spermatogenesis of the brittle-star *Amphipholis kochii* (Echinodermata: Ophiuroidea). *Publ. Seto Mar. Biol. Lab.* **28:** (in press).